

**REMARKS****I. Detailed Action**

A. Applicants acknowledge that the rejection of claims 10-12, 23-26, and 33-36 under 35 U.S.C. § 112, second paragraph is withdrawn in response to Applicants' arguments.

B. Applicants acknowledge that Applicants' arguments filed December 19, 2003 with respect to the rejection of claims 1-6, 8-11, 13-15, 16-18, 20-22, 24-25, and 38-39 under 35 U.S.C. 103(a) have been considered but are moot in view of the new ground(s) of rejection.

**II. Claim Rejections****A. 35 U.S.C. § 112, first paragraph**

Claims 13 and 32-37 stand rejected under 35 U.S.C. § 112, first paragraph as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner states that "the amount of experimentation necessary to determine the appropriate mode of delivery of the suppressor tRNA gene sequence into an animal, to access the possible toxicological effects the treatment may have on the translation of other known and unknown proteins in each cell in said animal is beyond the scope of one with ordinary skill in the art.." The Examiner further states that "although vectors may be available in the art that are able to deliver a gene to a particular tissue, the question remains regarding the production of the desired secondary effect in that particular tissue as a result of the expression of the gene of interest".

Applicants respectfully traverse this rejection. The test for enablement under § 112, first paragraph, is "whether or not the specification contains a sufficiently explicit disclosure to enable one having ordinary skill in the relevant field to practice the invention claimed therein without

the exercise of undue experimentation." Ex Parte Forman, 230 U.S.P.Q. 546 (Bd. Pat. App. & Int'l 1986).

As noted above, the Examiner has stated that vectors may be available to one of ordinary skill in the art which are able to deliver a gene to a particular tissue. Moreover, the specification teaches the use of any gene delivery vehicle known to skilled in the art. The specification extensively identifies several examples of such delivery vehicles and their efficacy. (Specification, p. 16-25).

The Examiner's main objection to the in vivo application and use of suppressor tRNA expression is, according to the Examiner, that the behavior of suppressor tRNAs with non-target genes is unpredictable and may result in secondary effects due to non-specific termination of protein synthesis that may affect all proteins that have the stop codon recognized by the therapeutic suppressor tRNA. Furthermore, the Examiner questions the assertion that the relatively low efficiency of the tRNA suppressor may not rescue the translation of nonsense mutations in target genes.

Applicants respectfully traverse the Examiner's assertions for the following reasons. First, the possible secondary effects which, according to the Examiner, may be "potentially devastating effects to a cell", are just an assumption based on overly simplistic and basic theories of protein synthesis and the genetic code. Second, there are innumerable examples in the literature where whole organisms (viruses, bacteria, fungi, yeast, insects, plants, algae, ciliates and mammals) live without any signs of toxicity in the presence of suppressor tRNA expression. In fact, multiple experiments involving the use of suppressor tRNA have been possible because they are not devastating to the host cell. As of the filing date of the present invention, one skilled in the art would have recognized that the relevant literature in the art supports this view. Applicants have

provided the Examiner below citations to several of these works of art, as well as information derived therefrom which would have been known to one skilled in the art at the time of filing. Moreover, subsequently developed pieces of art supports these viewpoints. Applicants have provided the Examiner with these citations and information derived therefrom as well.

There are multiple explanations as to why organisms can withstand the expression of suppressor tRNA. First, the dogma of the non-ambiguity of the genetic code does not hold for stop codons because these codons can have two or more meanings. In addition to mediating protein synthesis termination, stop codons are often involved in many protein re-coding strategies, such as protein readthrough, protein frameshifting, misincorporation of amino acids, incorporation of modified amino acids (i.e. selenocysteine) by natural suppressor tRNAs, modulation of protein synthesis by natural suppressor tRNAs, and nuclear nonsense mediated RNA degradation. For example, there is a natural and physiologic amount of protein readthrough by misincorporation of amino acids into stop codons, the extent of which is regulated by the sequence context surrounding the stop codon. Readthrough of nonsense codons UAG, UAA and UGA has been seen in *E. coli* strains that lack natural tRNA suppressors as well as in yeast and superior eukaryotic cells. For example, the UGA codon may mediate termination of polypeptide synthesis or trigger the incorporation of Trp, Arg or Cys. Likewise, the UAG termination codon may provoke the incorporation of Tyr, Glu or Leu.

Second, organisms express natural tRNA suppressors which affect certain stop codons but not all of them. In human cells there is a natural suppressor tRNA that incorporates selenocysteine in the UAA stop codon of glutathione peroxidase. This amino acid is not incorporated into every UAA stop codon of every protein demonstrating that specific sequence contexts and other factors besides the presence of the suppressor tRNA are involved in protein

synthesis termination and that the protein translation machinery has mechanisms to discriminate between different stop codons.

Third, there are no tRNAs that mediate protein termination. Protein synthesis termination is mediated by protein factors, ribosomal RNA loops and the primary and secondary structure of the mRNA being translated. In eukaryotic cells, translation termination involves an interaction between the release factor eRF1 and eRF3 and the stop codon within its nucleotide context. It is well known that the nucleotide context both upstream and downstream of the stop codon can modulate readthrough suggesting that complex interactions take place between the mRNA and the various components of the translation termination machinery in eukaryotes and prokaryotes. The precise molecular mechanism explaining the influence of downstream nucleotides and secondary structures on stop codon readthrough involves tRNA selection through stabilization of the tRNA-mRNA interaction by stacking effects, interaction between the stop codon and the rRNA, and interaction between the stop codon and the polypeptide chain release factor. Clearly, the competition between suppressor tRNA and the release factor is of great significance.

Fourth, there is a wide body of evidence that the stop codon of prokaryotes and eukaryotes is not just a trinucleotide but rather is a tetranucleotide with a strong influence on the sequence context and secondary mRNA structure at the termination codon. It has been demonstrated that the nucleotide following the stop codon (the +4 nt) is non random, with purines over-represented for the three stop codons. This +4 nt plays a key role in termination efficiency which is in fact inversely correlated with the ability to be suppressed by suppressor tRNAs. Other nucleotide biases are found around stop signals, non-randomness being observed at two to eight positions downstream and three nt upstream. Highly expressed genes show clear preferences among stop codons and their contexts, whereas inefficient stop signals are exploited

in a variety of recoding processes involving natural suppressor tRNAs. Where tRNA suppressors of nonsense codons are present, both the efficiency of suppression and of the termination process appear to be affected by stop codon context. The two bases downstream of the nonsense codon and the nature of the contiguous upstream codon are major determinants of suppression efficiency. The influence on the sequence context surrounding the stop codon creates a distinction between natural stop codons and premature nonsense mutant stop codons (which have not evolved an optimal sequence context for protein termination), making the premature nonsense stop codons more suppressible than natural stop codons. Although natural suppressor tRNAs differ in their efficiency to recognize leaky stop codons (suppressible stop codons) in various codon contexts, none of them are capable of misreading genuine stop codons at the end of natural open reading frames, thus providing an explanation for the lack of cytotoxicity associated with the expression of suppressor tRNAs at normal levels.

Fifth, the efficiency of the suppression of protein synthesis termination correlates with the levels of expression of the suppressor tRNA and on the post-synthesis modification of bases of the tRNA loops (natural or artificial). This means that low levels of expression of suppressor tRNAs will lead to low levels of suppression and protein readthrough, which will not affect the function of normal proteins. In many settings where a therapeutic suppressor tRNA is used to promote the readthrough of premature nonsense codons, a low percentage of suppression of the mutant protein is enough to restore the mutant phenotype. For example, expression of coagulation factor IX to 1% of normal levels is enough to control coagulation abnormalities in hemophiliacs. Similarly, expression of adenosine deaminase to 5% of normal levels is enough to control the immunodeficient phenotype in ADA patients. Likewise, expression of 30% of normal dystrophin would be enough to restore the Duchenne and Becker muscular dystrophy.

Therefore, there are numerous applications where a low level expression of a suppressor tRNA could be useful in therapeutic applications with minimal risks for secondary effects.

Finally, there is direct evidence that argues against a generalized cytotoxic effect resulting from expression of suppressor tRNA exists in many organisms. The most simple system are bacteria expressing suppressor tRNAs, which are perfectly viable. Also, mammalian cells in culture that express high levels of suppressor tRNAs do not display toxic phenotypes. Also, supersuppressor strains of *S. cerevisiae* and *N. crassa* are able to grow without abnormalities, and *in vivo* expression of suppressor tRNAs in mice does not result in toxic effects while resulting in measurable suppression of nonsense codons. Applicants have described some cytotoxicity associated with the expression of suppressor tRNA expressed from HSV vectors. This cytotoxicity is typical of HSV vector preparations which often consist in a mixture of vector and helper virus which has its demonstrated cytotoxicity. Control HSV vectors preparations that do not express suppressor tRNA display the same degree of cytotoxicity as the one seen for vectors expressing the sup tRNA. Moreover, expression of sup tRNAs into mammalian cells after transfection of plasmids bearing multiple copies of sup tRNA does not result in measurable cytotoxicity. Therefore, the cytotoxicity described in Applicants' specification is not the result of sup tRNA expression.

In support of Applicants' arguments, see Tate et al, 1996, "Hidden infidelities of the translational stop signal", *Prog Nucleic Acid Res Mol Biol*, 52: 293; Buvoli et al, 2000, "Suppression of nonsense mutations in cell culture and mice by multimerized suppressor tRNA genes", *Mol. Cell. Biol.* 20:3116; Arkov et al, 1995, "5' contexts of *Escherichia coli* and human termination codons are similar", *Nucleic Acids Res.* 23: 4712; Brown et al, 1990, "Sequence analysis suggests that tetra-nucleotides signal the termination of protein synthesis in eukaryotes",

Nucleic Acids Res. 18: 6339; Brown et al, 1990, "The signal for the termination of protein synthesis in prokaryotes", Nucleic Acids Res 18: 2079; Kuchino et al, 1996, "Nonsense suppression in mammalian cells", Biochimie 78: 1007; Tate et al, 1996, "The translational stop codon signal: codon with a context or extended factor recognition element?", Biochimie 78: 945; Beier et al, 2001, "Misreading of termination codons in eukaryotes by natural nonsense suppressor tRNAs", Nucleic Acids Res. 29: 4767; Cassan et al. 2001, "UAG readthrough in mammalian cells: effect of upstream and downstream stop codon context reveal different signals", BMC Molecular Biology 2:3; Namy et al. 2001, "Impact of the six nucleotides downstream of the stop codon on translation termination", EMBO J Sept 2001, 2:787; Buckingham et al, 1990, "Third position base changes in codons 5' and 3' adjacent to UGA codons affect UGA suppression in vivo", BB Acta 1050:259; Buckingham et al, 1990, "Codon context", Experientia 46: 1126; Firoozan et al, 1991, "Quantitation of readthrough of termination codons in yeast using a novel gene fusion assay" Yeast 7: 173; Nilsson et al, 2003, "Glutamine is incorporated at the nonsense codons UAG and UAA in a suppressor free E. coli strain" BB Acta 1627:1; and Ganoza et al, 1984, "Effect of base sequence on in vitro protein chain termination" J. Biol. Chem. 259: 14101.

Therefore, Applicants respectfully submit that claims 13 and 32-37 are enabled and in condition for allowance and therefore request withdrawal and reconsideration of the claims rejected under 35 U.S.C. § 112, first paragraph. Accordingly, withdrawal of the rejection is requested.

*B. 35 U.S.C. § 112, second paragraph*

Claims 1-27 and 29-38 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

With regard to claims 1-27 and 29-38, the Examiner states that "it is unclear if the anticodon region is the same as the anticodon sequence, or do they represent separate components of the oligonucleotide sequences of the claimed invention." Applicants have amended the claims to clarify the distinction between anticodon region and anticodon sequence. Applicants therefore submit that claims 1-27 and 29-38 are in condition for allowance.

With regard to claims 1-27 and 29-38, the Examiner states that "it is unclear if the limitation 'a total length of less than 150 nucleotides' refers to the total length of the oligonucleotide sequences of the claimed limitation or does this limitation refer to the total length of the suppressor tRNA." Applicants have accordingly amended the claims and therefore submit that claims 1-27 and 29-38 are in condition for allowance.

With regard to claims 17-18 the Examiner states "there is insufficient antecedent basis for this limitation in claim 14. Claim 14 is drawn to a nucleotide vector." Applicants have amended claims 17 and 18 so that there is sufficient antecedent basis. Applicants wish to thank the Examiner for pointing out this inadvertent mistake.

With regard to claims 29-31 the Examiner states that "there is insufficient basis for this limitation because claim 28 has been canceled." Applicants have canceled claims 29-31. Applicants wish to thank the Examiner for pointing out this inadvertent mistake.

Therefore, in view of the amendments made to claims 10-11, 23-25, 33-35, Applicants believe that the claims are allowable under 35 U.S.C. 112, second paragraph. Accordingly, withdrawal of the rejections is requested.

*C. 35 U.S.C. § 103*

Claims 1-6, 8-11, 13-15, 16-22, 24-25, and 38-39 stand rejected under 35 U.S.C. 103(a) as being unpatentable under Sharp et al, Temple et al, Li et al and Noren et al in view of Atkinson et al and Capone. Applicants respectfully traverse this rejection.

The Examiner has the burden under 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988). The test under § 103(a) is whether the differences between the prior art and the invention, as a whole, would have been obvious to one having ordinary skill in the art. *Ruiz v. A.B. Chance Co.*, 234 F.3d 654, 662 (Fed. Cir. 2000). The prior art must teach one of ordinary skill in the art to combine elements from the prior art in the manner combined by the inventor. *Crown Operations Int'l., Ltd. v. Solutia, Inc.*, 289 F.3d 1367, 1376 (Fed. Cir. 2002). All of the claim elements must be taught or suggested by the combined references. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Thus, obviousness can not be determined by a hindsight gathering of elements in order to "fit the parameters" of the invention. *ATD Corp. v. Lydall, Inc.* 159 F.3d 534, 546 (Fed. Cir. 1998).

The Examiner states that "it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the instant application to design oligonucleotides encoding human suppressor tRNAs of less than 150 bp and to design methods of use of said human suppressor tRNAs embraced by the claimed invention. One of skill in the art at the time of the instant

invention would have been motivated to combine the cited references in the design of the claimed invention because all of the references cited teach various aspects in regards to the preparation and design of suppressor tRNA molecules."

The Examiner states that Sharp et al. teaches a "general method of designing and using suppressor tRNAs to by-pass nonsense mutations in proteins in mammalian cells" and notes that Noren et al. "teach[es] a method of site specific incorporation of un-natural amino acids into proteins, wherein said method comprises replacement of a codon encoding an amino acid of interest, replacing the codon with a nonsense codon (TAG) by oligonucleotide directed mutagenesis, and designing a suppressor tRNA chemically aminoacylated in vitro to recognize the nonsense codon and direct the amino acid into the protein at the target site." The method taught by each of these references involves isolating, cloning and finally site-mutating the tRNA molecule to alter the anticodon. In contrast, the present invention as described by the claims teaches a novel two-step method that involves designing an oligonucleotide containing only the tRNA structural sequence and less than 20 3' flanking nucleotides that incorporates the desired alteration of the anti-codon, followed by synthesis of the oligonucleotide. As noted by the Examiner, neither of these references teach the synthesis of an oligonucleotide with a total length of less than 150 nucleotides and with no more than twenty 3' flanking residues and no 5' flanking residues, elements contained in independent claims 1, 7, 8 and 22.

The Examiner next notes that "Temple et al. disclose a functional human lysine tRNA (containing an anticodon which recognizes the amber termination codon UAG) gene whose length is approximately 76 base pairs, this gene was subcloned into M13mp7 phage." The functional human lysine tRNA taught by Temple is contained within a subcloned tRNA gene fragment that is 800bp in length. The Examiner further notes that "Li et al teach[es] the use of a

human suppressor serine tRNA which has functions in the rescue of mdx (gene associated with Muscular Dystrophy in humans) gene expression lost due to an Ochre (UAA) mutation." As noted by the Examiner, neither of these references teach the synthesis of an oligonucleotide with a total length of less than 150 nucleotides and with no more than twenty 3' flanking residues and no 5' flanking residues, elements contained in independent claims 1, 7, 8 and 22.

The Examiner then states that Atkinson et al. teaches that "the length of an active transcription unit of a tRNA gene may be considerably less than 500 base pairs so that accommodation into a delivery vector may be facilitated." However, Atkinson et al. does not teach the synthesis of an oligonucleotide with no more than twenty 3' flanking residues and no 5' flanking residues, elements contained in independent claims 1, 7, 8 and 22. Further, while Atkinson et al. does suggest that the transcription unit of a tRNA gene may be less than 500 base pairs, it does not suggest that the synthesized oligonucleotide may be as small as 150 base pairs. This feature of the present invention facilitates cloning and allows the incorporation of multiple (tandem) copies of the tRNA-coding oligonucleotide in the vector, and is a necessary element contained in independent claims 1, 7, 8 and 22.

Finally, the Examiner states that "Capone teaches that complete removal the 5' flanking sequence does not prevent the modified serine tRNA from functioning as a suppressor tRNA. Additionally, it is noted that serine tRNA mutants comprising only 22 bp of 3' flanking sequence still maintained suppressor activity that was about 30% less than wild-type." While Capone does teach the functional result of modified serine tRNA with 22 base pairs of 3' flanking residues, it again does not contain the necessary elements as claimed by independent claims 1, 7, 8 and 22. Capone does not suggest that functional oligonucleotide sequences may be synthesized which are

only 150 base pairs in length and which have only twenty 3' flanking residues, and no 5' flanking residues.

None of the individual references cited by the Examiner render Applicants' invention obviousness to one skilled in the art. Moreover, even if the references are combined, they do not teach one of ordinary skill in the art to combine elements from the prior art in the manner combined by the inventor. As noted by the Examiner, Sharp et al., Noren et al., Temple et al., and Li et al. do not teach an oligonucleotide encoding a synthetic suppressor tRNA wherein the oligonucleotide is less than 150 base pairs in length and has no 5' flanking residues and less than 20 3' residues. The same is true for Atkinson et al. and Capone. While Atkinson teaches the use of an oligonucleotide with a shortened base pair length and Capone teaches that only 22 3' flanking residues are necessary and that the 5' flanking residues may be removed from an oligonucleotide, neither of these references teach the elements of the Applicants' invention- the synthesis of an oligonucleotide which is less than 150 base pairs in length and has no 5' flanking residues and less than 20 3' residues. Further, there is no suggestion within the references that these elements may be combined in the manner done by the Examiner. None of the cited references suggest or teach that a functional oligonucleotide can be synthesized which is both, at the most, only a 150 base pairs long and, at the most, has only 20 3' flanking residues, in addition to no 5' flanking residues. Therefore, Applicants respectfully submit that claims 1-6, 8-11, 13-15, 16-22, 24-25, and 38-39 are nonobvious and in condition for allowance and therefore request withdrawal and reconsideration of the claims rejected under 35 U.S.C. § 103(a).

Claims 16-18 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Sharp et al., Temple et al., Li et al., Noren et al., in view of Atkinson et al. and Capone as applied above and in further in view of Okasinski. Applicants successfully transverse this rejection.

The Examiner notes that "[n]one of the above references [Sharp et al., Temple et al., Li et al., Noren et al., Atkinson et al. and Capone] teach the use of an HSV vector comprising a nucleotide encoding a human suppressor tRNA molecule according to claim 1 of the instant application." Sharp et al, Temple et al, Li et al, Noren et al, Atkinson et al and Capone are discussed above. As noted, none of these references, alone or combined, teach the synthesis of functional oligonucleotide sequences which are only 150 base pairs in length and which have only twenty 3' flanking residues, and no 5' flanking residues.

Okasinski does not teach the missing elements of Sharp et al, Temple et al, Li et al, Noren et al, Atkinson et al, and Capone. As stated by the Examiner, Okasinski discloses "an eukaryotic expression vector containing HSV DNA and regulatory elements, and sites for subcloning a DNA of interest. In addition, Okasinski discloses methods of producing a mammalian cell line having cells containing the expression vector." There is no motivation in the cited references to combine the teachings of the references so as to arrive at the claimed invention- the synthesis of functional oligonucleotide sequences which are only 150 base pairs in length and which have only twenty 3' flanking residues, and no 5' flanking residues. Therefore, Applicants respectfully submit that claims 16-18 are nonobvious and in condition for allowance and therefore request withdrawal and reconsideration of the claims rejected under 35 U.S.C. § 103(a).

### III. Conclusion

In light of the above remarks, Applicants respectfully assert that claims 1-27 and 29-38 are now in condition for allowance. Applicants respectfully request reconsideration and withdrawal of the above rejections.

This is a request under the provision of 37 CFR § 1.136(a) to extend the period for filing a response in the above-identified application for one month from June 10, 2004 to July 10, 2004. Applicants are a small entity; therefore, please charge Deposit Account number 26-0084 in the amount of \$55.00 for one month to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to Deposit Account 26-0084.

No other fees are believed to be due in connection with this amendment; however, consider this a request for any inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,



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